Mechanisms of Microbial Pathogenicity in the Human Gut

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Bacteria, viruses and fungi are all normal inhabitants of the human intestine [1,18]. Many of the bacteria may be opportunist pathogens elsewhere and in the gut whereas others are pure commensals. Viruses are found but comparatively little is known of those responsible for disease in situ.

In this essay an attempt will be made to examine the sources of infection, host anti-bacterial mechanisms, factors important in bacterial residency and modes of bacterial pathogenicity. Fungi and protozoa are not considered in this essay and viruses are only briefly examined.

It may be useful to indicate the classes of bacteria which occur and their normal position in the gut since deviation from this position may cause disease.

Gorbach, et al [22] have demonstrated two distinct microbial ecologies in the human gut. The small intestine consists of small numbers (up to 10^5) of streptococci, lactobacilli, staphylococci and fungi, their numbers increasing towards the distal end of the ileum. It has been suggested that the ileocaecal valve demarcates the two ecologies since beyond this, bacteroides, anaerobic lactobacilli and coliforms predominate (up to 10^{12} per gram of tissue). The distal end of the ileum may be considered as a transitional zone since it has a modified faecal flora.

Previously, the ileocaecal valve was thought to be responsible for the demarcation of the two distinct groups. However, work by Percy-Robb and Collee [38] suggests that the ionization of bile acids may be responsible in preventing retrograde colonization of the upper gut. Using strains of Clostridia species and Bacteroides species which both deconjugate taurocholic acid efficiently, they showed that unconjugated bile acids have a bactericidal or bacteriostatic effect, which is pH dependent. The maximum bactericidal effect occurs at pH 5.8 with concentrations of bile acid grater than or equal to 1.5 mmol/l. Northfield et al [37] have demonstrated free bile acids in the terminal ileum and these figures are compatible with the normal gut pH and bile acid concentration here. At concentrations less that this, unconjugated bile acids may exert a bacteriostatic effect.

Work by Meynell [33,34] has shown that there are free volatile fatty acids in the caecum and he suggests that these too may be involved in the segregation of the two ecologies or in a bactericidal mechanism.

Before any microbe can be entero pathogenic it must first reach the part in the gut whence it orients its pathology. It seems logical therefore to consider sources, and mechanisms which may be involved in the colonization of the gut by this microbe or the mechanisms by which the host may defend itself from a potentially pathogenic species. The bacterial population of the gut is dynamic and new strains may be acquired from a number of sources but food as a source is of prime importance. Shooter and Cooke, [42] found large numbers of E. coli animal strains in hospital food, in the hospital kitchen, on meat on arrival at the kitchen, and in the abatoir itself. By this route, animal strains may reach the human population, and although they are not as viable as human strains in the human gut, they may carry RTF and may thus be of importance in treating subsequent genito-urinary tract infections due to E. coli. serotypes.

Contamination occurs mainly in the washing-up process. Marked contamination of dish water was found and the authors noted that milk and semi-solid foods were important carriers of infection. When plates which were not adequately washed were left to dry, they were found to be good media for the bacteria to grow on. This correlated with a previous observation that semolina, milk and blancmange were potential sources of E. coli in the ward.

Cooke, E.M. [5,6] has shown that there is no dominant strain of E. coli in hospital medical patients and that the number of serotypes observed depends on the number of specimens examined. Patients in the ward ate food containing large numbers of E. coli and this accounts for the changing gut flora. Ingestion of 10^4 bacteria or more results in colonization for 2-8 weeks. Frequently patients swallow more than this and at no time was a strain found in food which was not subsequently found in the faeces. The diet as a source of new strains was confirmed when 5 patients acquired fresh serotypes after eating food containing them. In support of this, Kennedy has shown that certain 'O' serotypes of E. coli are more common in hospital patients than the general public. Cooke also showed that types isolated by them were responsible for many genito-urinary infections in the ward. The hospital food probably explains the acquisition of these new serotypes.

Gut commensals may be resident or transient and this may involve

colicine production although host factors seem to play an important role. Branche, et al [2] in a study of faecal samples from 6 patients over a period of 6 months noted the association between multiplicity of E. coli serotypes during this period and little or no colicine production as compared with few serotypes and colicine production. He concluded that residnecy is associated with colicine production and that transient strains do not produce colicines. However, Cooke, et al [7] could find little evidence to agree with the hypothesis that colicine production by a particular strain conferred an absolute advantage over susceptible strains in acquiring an ecological niche, although on occasion it was noted that non-colocine-producing strains were displaced by colicine-producing ones. Ineffectivity in vivo may be due to a variety of causes.

1) The sensitive strain may develop tolerance. It was demonstrated by Richardson, et al [39] that tolerance may be induced in vitro by exposing the sensitive strain to low concentrations of colicine while the culture is in the exponential phase. According to the author, tolerance develops due to some organisms absorbing a sub-lethal amount of the colicine which either induces a conformational change in the membrane or initiates a repressor to block the colicine's effect on a particular biochemical target. If tolerant cells are treated with trypsin, there is a restoration of the sensitivity but the colicine is retained at the receptor. This explains the decreased rate of killing on the second exposure to the colicine after trypsin digestion.

2) The colicine may be labile in the intestinal environment.

3) Colcinogenic strains may concommitantly produce an inactivator. This is supported by in vitro observations where colicine activity was observed after the first 24 hours but not after 48 hours.

4) Intestinal concentrations of the colicine may be inadequate to inhibit other strains. This may be due to proteolytic degradation of the colicine, inactivation by an inactivator, its labile nature or to Resistance Transfer Factors. Kromery [29] has shown that a col factor may be eliminated from colcinogenic E. coli strains after infection by certain RTF. These factors appear to inhibit colicine production but it was noted that not all col factors were equally susceptible to elimination by R factors. Arai et al has demonstrated that resistance may be induced due to inactivation of the colicine by an RTF mediated enzyme.

Cooke et al [7] has shown that host factors play an important role in colonization of the gut by bacteria. Previous authors had shown that it was difficult to introduce new strains. However, Cooke and her co-workers have shown that milk is very successful and may be used to introduce animal strains also. In this experiment, 3 subjects with an apparently normal gastro-intestinal tract were infected with one of twelve strains of E. coli of animal and human origin at different times over several months. These strains were of known serotype, antibiotic sensitivity, colicinogenicity, and colicine sensitivity. The authors showed that the strain may be recovered in the faeces, and that only in 4 cases in 25 experiments did the strain persist for longer than one month. However, subject variation is very important, since on three occasions, different strains persisted in the same subject and in only one case did one of these strains persist in another subject. When 10^5 to 10^8 bacteria were introduced it was observed that human strains colonize more effectively than animal ones. However, when 10^{11} organisms of an animal strain were introduced they persisted for up to 120 days. A human is unlikely to consume as many as this under normal circumstances.

Smith [44] had previously observed that animal strains are poorer at colonizing the human alimentary tract than human ones, and that larger numbers of bacteria were necessary to colonize the gut. He did not observe that there was significant spread of R factors throughout the gut from ingested strains of E. coli to resident strains as was anticipated. He also noted that those strains which did gain resistance did not persist, although he did confirm that R transfer does take place from animal strains to resident strains in the human gut. He concluded that the large amount of antibiotic resistance observed in the community was not primarily due to ingested animal strains possessing RTF colonizing the human alimentary tract and conferring resistance upon resident strains. However, he did point out that this system may be important in farm workers. Indeed, ampicillin resistant strains were isolated from two farm workers who dealt with intensively reared calves also bearing these strains.

One criticism levelled at Smith's work is that his experiments were all performed on one subject, and as Cooke points out, host variability is very important when considering colonization of the human intestine.

Since some ingested strains of E. coli are toxigenic, they may cause

diarrhoeal syndromes. The possession of the K88 antigen by these strains may enhance their pathogenicity. K antigens are capsules or envelopes and, according to Jones and Rutter [28] the K88 antigen enables strains which possess this to adhere more successfully to a mucosal surface in vivo than those strains which do not possess it. Using fluorescent techniques and an antiserum to K88 they demonstrated that $K88^+$ strains adhere to the mucosa of both the small and large intestine. However, using three $K88^-$ mutations prepared in different ways they found that these were randomly distributed throughout the lumen of the small intestine although they did adhere to the mucosa of the large intestine. In vitro observations confirmed the $K88^+$ cells adhered to the small intestine mucosa of gnotobiotic pigs. After oral dosing, wild type $K88^-$ mutants attached to both large and small intestine mucosae although they adhered less well than $K88^+$ cells. Fimbriae do not appear to be involved in the mechanism of adhesion, since no mannose-sensitive haemagglutination was observed.

The K88 antigen may be considered as an adaption to a moving environment which gives close proximity to nutrients. Evidence from starved pigs suggests that E. coli may utilize mucus, but since mucus removes particles from the gut, E. coli must penetrate the mucus to attach to the epithelium. This possibly explains the observed glycosidase production by these strains. However, it has been observed that human enteropathic strains have a higher mucinolytic activity than non-enteropathic strains. The authors point out that porcine intestine does not react similarly to human or pig intestine with this mucinase and hence should no longer be considered a viable test for mucinase activity.

In gnotobiotic pigs, $K88^+$ and $K88^-$ strains were equally pathogenic and were persistent in the pig gut due to stomach colonization and the reduced motility. This indicates that attachment was not essential for enterotoxin production and subsequent enteritis. However, in normal pigs, K88 was essential for virulence of the strain and subsequent pathology.

In humans the capsule appears to act as a virulence factor protecting the bacillus from phagocytosis, and may possibly be involved in attachment of the bacillus to the mucosal epithelium.

Other factors which are involved in the colonization of the gut include the host's own antibacterial mechanisms. One such mechanism, the pH dependent bile acid system has been discussed with reference to separation of the small and large intestinal flora. Other mechanisms include the effect of normal microbial flora and the bactericidal effect of gastric juice.

The effect of normal flora may be summarized as competition for available nutrients and the inhibiting effects on other species or strains of colicine production by colicinogenic strains. Hentges and Freter [25] have shown that the most potent inhibitor of other strains *in vitro* is *Proteus vulgaris* but *in vivo* *E. coli* is the most important. The authors point out that colicine production is usually observed in aerated colonies and that the intestine is strongly reducing. Hence, their methods of continuous flow culture may illustrate the main mechanism of antagonism but colicines may still be of primary importance in the human gut, since it has been demonstrated by Young et al [48] that colicine production may be induced in non-producing colcinogenic streams. These induced colicines may have a role in the ecology of the intestinal flora since a particular strain may produce colicine under unfavourable environmental conditions.

In the following paper Hentges and Freter [25] showed that competition for fermentable carbon sources is involved in the antagonism of certain strains. This system is probably of major importance *in vivo* in limiting the growth of pathogenic strains in the small intestine. Work in gnotobiotic animals demonstrates this. When a bacterial species is introduced it rapidly colonizes since there are no species opposing it, and rapidly exerts its pathogenic effect if any. In the normal animal, colonization may be inhibited by the presence of other organisms competing for the same carbon source.

Using an abortive transductant as a genetic marker, Meynell and Subbiah [33] determined the number of bacterial divisions in normal mouse gut and streptomycin treated mice. Since the division rates in normal gut were less than in the streptomycin treated mice, they concluded that normal mouse gut contains a bacteriostatic and weakly bactericidal mechanism. The authors considered that this mechanism was weak as

streptomycin abolished its effects. Streptomycin acts by inhibiting growth of anaerobes (Bacteroids and Lactobacilli) which are responsible for maintaining an acid pH since they produce short chain fatty acids. Meynell [34] considered that these volatile fatty acids and the associated low negative redox potential were important. He justified this by showing that *in vitro* a low redox potential and appreciable quantities of volatile fatty acids inhibited *Salmonella typhimurium* and that as the free fatty acid concentration decreased, thus raising the redox potential, *Salmonella typhimurium* were less inhibited.

In work with *Shigella sonnei* and natural and synthetic gastric juices Dare et al [9] have shown that *in vitro* as pH decreases below 3, there is an increasing lethal effect on this bacillus. The major bactericidal component is the acid but this is enhanced greatly by pepsin lysozyme and amino acids contribute to the lethal properties of the artificial system, but sugars and organic acids protect the bacillus. IgA is present in natural gastric juice and this also has a role in protecting the host, provided that this microbe has been encountered previously.

Bacteria may cause disease in many ways including overgrowth, toxin production, invasion of the mucosal epithelium and possibly conversion of bile acids into potent carcinogens.

The most common finding in small bowel bacterial overgrowth is megaloblastic anaemia due to vitamin B_{12} deficiency. This is due to uptake of the vitamin in the proximal small bowel by bacteria causing less to reach the ileum where it is absorbed. In a review, Donaldson [10] pointed out that Gram negative bacteria bound the labelled vitamin and intrinsic factor complex to a degree although intrinsic factor appeared to slow down the bacterial uptake of the vitamin.

Due to the increased numbers of bacteria present in small bowel bacterial overgrowth, increased quantities of bile acids are deconjugated causing lipid malabsorption and steatorrhea. Bacteria may also desaturate and hydroxylate fatty acids from ingested lipid changing it so that it cannot be metabolised normally. Associated with lipid malabsorbtion is a deficiency of fat soluble vitamins eg. D and K which may result in further pathology. Folate deficiency is not seen in overgrowth since some bacteria synthesize and release folic acid.

Bacterial overgrowth may result from decreased peristalsis due

to vagotomy or to overgrowth in diverticula. In both these situations, the bacteria are in a slower moving or stationary environment which does not slough them off. Failure to separate the two florae may cause bacterial overgrowth. Eg. Normal colon commensals like E. coli may outgrow the normal flora of the small intestine if the separation breaks down.

In small bowel bacterial overgrowth, in general, no particular species outgrows the others. There is a progressive increase in numbers of all of the species although this is limited eventually by competition and host defence mechanisms.

Bacteria may cause disease by producing toxins. These may be produced extracellularly and difruse, or are transported into, the cell where they exert their effects. Vibrio cholerae produces an enterotoxin of this type. Alternatively, the bacterium may enter the cell, multiply here, possibly produce toxins which have their effects on this cell and others nearby. Shigella dysenteria probably produces a toxin of this type.

A considerable amount of work has been performed by Duncan et al [11-16] on the nature and time of production of enterotoxin by Clostridium welchii Type A (Cl. perfringens). This bacillus causes a mild form of food-poisoning typified by abdominal cramps and diarrhoea with no vomiting which gets better within 72 hours.

Using a medium on which the bacillus sporulates, he has demonstrated that toxin production is intimately related with spore production. Enterotoxin production lagged the spore formation by about 2½-5 hours but thereafter the concentration increased with the increasing number of spores. Extracellular toxin was observed after 10 hours and was coincident with the first release of free mature spores. From this, Duncan deduced that enterotoxin was released on lysis of the sporangium. Concomitant with spore production an inclusion body is formed in the bacillus. This may be demonstrated by phase-contrast and electron microscopy and appears to resemble a crystalline structure. In experiments with enterotoxin producing strains (ent $^{+}$) and non-producing mutants (ent^{-}) he has demonstrated that only ent^{+} strains show the inclusion during sporulation, and that a single gene is responsible for sporogenesis, enterotoxin synthesis and ability to form an inclusion.

Duncan has further observed that purified enterotoxin undergoes aggregation at concentrations greater than 4 mg/ml and during ultra-centrifugation. This suggests that the inclusion is an accumulation of enterotoxin or of an enterotoxin subunit, which is distinct from an over produced spore coat although it may be related to the spore coat protein.

The enterotoxin is heat labile, non-dialysable and cell free enterotoxin may be detected by fluid accumulation in ligated rabbit ileal loops. This was confirmed by Hauschild [23]. The toxin is not neutralized by antitoxins to toxins of Cl. welchii strains, A,B,C,D or E and immunodiffussion studies with polyacrylamide disc electrophoresis indicate that the toxin is a single protein of molecular weight between 34 and 35 x 10^3 Daltons. Confirmation that a single protein was responsible for the observed fluid accumulation followed from chromatographic separation of the toxin, where the major peak was shown to be responsible for the enterotoxin's activity. A small 280/260 ratio was also observed which is indicative of a nucleic acid possibly responsible for the erythremal activity observed in mice or in inclusion formation.

Hauschild has purified and identified the enterotoxin and his results are largely comparable to those of Duncan above.

In monkeys, strains which produced fluid accumulation in the ileum also caused vomiting, the symptoms arising between 4½ and 21 hours after ingestion of the bacillus.

Some symptom producing and non-symptom producing strains in the rabbit ileum were subsequently tested for enteropathogenicity in humans [16]. A challenge dose was prepared by growing the bacillus in beef stew for 3 hours at 46 C. 61% of the strains which produced symptoms in rabbits also did so in humans, and all strains which produced no symptoms in rabbits, produced no symptoms in humans. When a cell free filtrate extracted from the broth of rabbit positive strains was administered, 4 out of 15 subjects developed symptoms. Again, strains which produced no symptoms in rabbits, did not produce symptoms in humans. The degree of subject suggestibility was not investigated although it was observed that there was an enhanced response if a lot of food was eaten

with the inoculum probably due to the buffering effect on gastric acidity.

Hauschild et al [24] concluded that the toxin from Cl. welchii Type A was responsible for the diarrhoea and abdominal pain in an outbreak of food-poisoning in humans. He further noted that although one strain would not sporulate on the sporulating medium, it did so readily in the human gut causing the above symptoms.

Having examined how toxin may be produced in Cl. welchii Type A, it seems appropriate to examine how this toxin may act. A considerable amount of evidence has built up on how the enterotoxin from Vibrio cholerae acts.

This toxin like that of Cl. welchii is a protein with a molecular weight of about 84×10^3 and is very potent at producing fluid accumulation in the gut even after a brief exposure followed by repeated washings. Previously it was thought that the fluid accumulation was due to an acute inflammation in the small intestine. However, intestinal biopsies and subsequent light and electron microscopy have demonstrated and apparantly normal intact surface epithelium.

The fluid which accumulates is isotonic with plasma, contains very little protein but has a higher bicarbonate, chloride and potassium concentration than normal.

Field, Plotkin and Silen [19] first demonstrated that when cyclic 3'5' adenosine monophosphate (cAMP) concentration was increased in the rabbit ileum both by inhibiting its breakdown by phosphodiesterase with theophylline, and by addition of cAMP, this caused an increased secretion of chloride and bicarbonate ion into the gut lumen. From this observation, they considered that cholera toxin may act by stimulating adenyl cyclase to produce cAMP. This was confirmed when it was observed that, following toxin administration, theophylline had no further effect on fluid accumulation.

Three mechanisms may account for the fluid accumulation;

i) it may be due to a failure of absorbtion,

ii) there may be increased filtration, or

iii) it may arise from increased secretion which is energy dependent.

The first mechanism may be discounted since it has been observed that glucose absorption is not impaired and that the associated sodium flux into the mucosal cell is unaltered in the presence of toxin.

For increased filtration, there would have to be an increased hydrostatic pressure or increased permeability. Fordham has demonstrated that intracapillary pressure was so low, it could not account for the exsorption and that although increased serosal pressure would have the same effect, this is of unlikely significance in the pathogenesis of cholera. If the capillary and mucosal cell permeability were increased, one would expect to find an exudate with a significant protein content and this is not observed. It has also been noted that a 30% decrease in blood flow has no effect on fluid exsorption. The role of increased permeability seems to be an unlikely cause of the fluid accumulation.

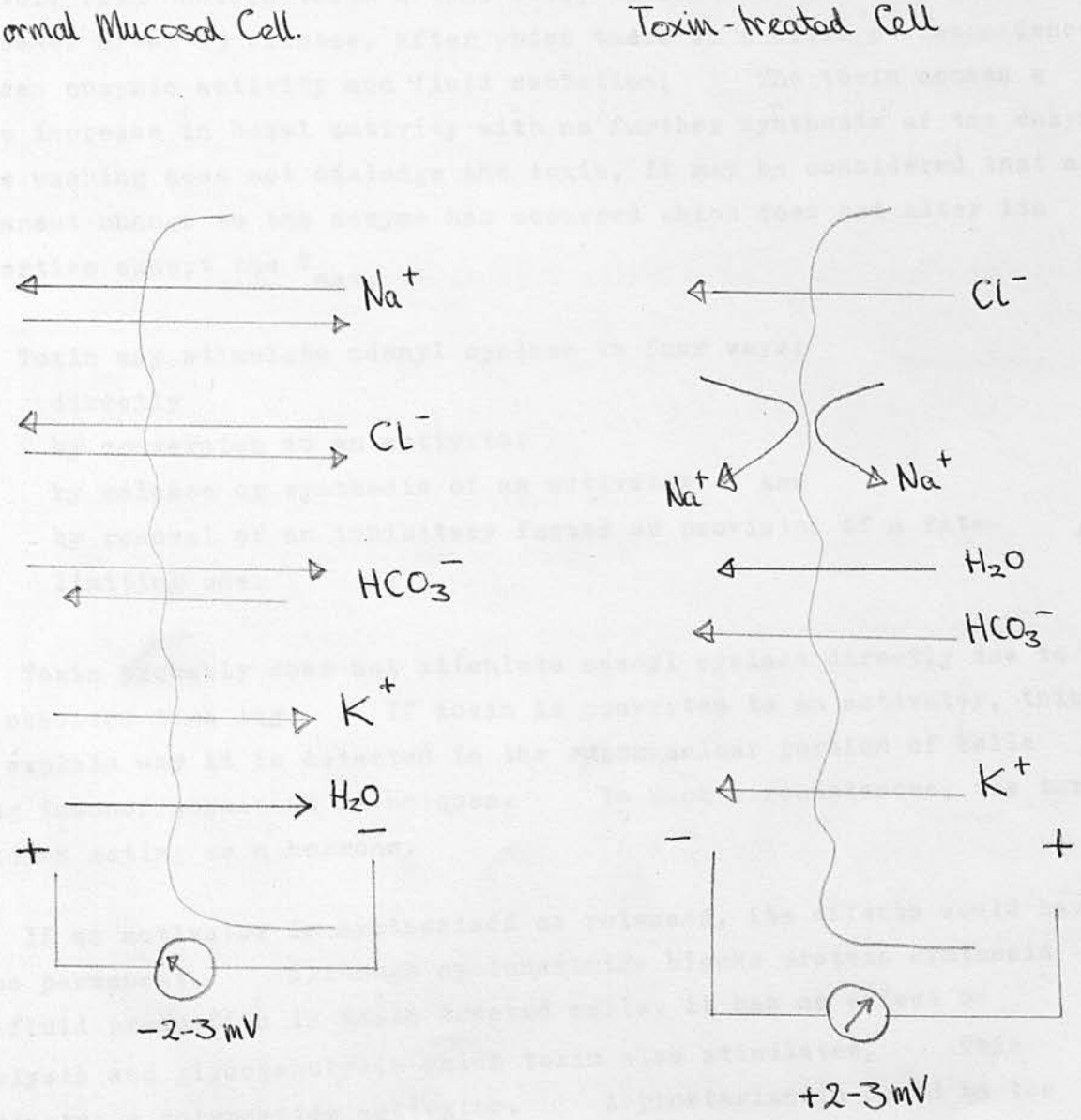
Increased fluid secretion appears to be the mechanism by which the toxin exerts its effects [40,41,42]. Different areas of the intestinal mucosa are responsible for absorption and secretion. The villous columnar epithelium is responsible for absorption and the crypts for secretion. Cycloheximide, which arrests cell mitosis in the crypts of the mucosal cell inhibits the action of the toxin, whilst not effecting glucose absorption. Hence, the toxin exerts at least part of its exuding action here. Sine Field et al has demonstrated that cholera toxin causes an increased intracellular concentrate of cAMP it probably mediates its effects on the cells in the crypt by this mechanism. It has been shown by the fluoride technique that no further synthesis of adenyl cyclase occurs whilst the toxin is present, so it does not cause increased cAMP concentrations by virtue of increased adenyl cyclase concentrations in the cell membrane.

Fluid loss may be reduced by administration of ethacrynic acid which is a potent inhibitor of adenyl cyclase, thus confirming that this is involved in the mechanism of fluid loss. Cholera toxin may stimulate adenyl cyclase in other situations including fat cells, skin capillaries, hepatocytes and platelets.

Toxin stimulates anionic secretary processes into the lumen of the small intestine in excess of the resorptative capacity. Using short-circuit techniques, results of which are debatable, since they only moniter

overall changes, conflicting evidence on actual ion movement has been gathered. Field et al [21] considered that chloride ion secretion was the major response to toxin <u>in vitro</u> whereas Moore [35] considers bicarbonate ion to be of major importance <u>in vivo</u>. Field has subsequently acknowledged that bicarbonate ion is the predominant ion flux <u>in vivo</u>.

Moore et al [35] have shown that water absorption decreases after two hours following the challenge with toxin and that after four hours, there is water secretion. This change is associated with increased potassium and bicarbonate ion concentration in the recirculating fluid and a change in overall potential difference from a few mV positive to a few negative. A proposed mechanism of ion movements in normal and toxin treated mucosal cells is illustrated below.



Potassium ion diffusion takes place into the lumen due to the negative intraluminal potential difference. However, potassium concentration is 2 to 3 times that expected from the Nernst equation implicating an active secretary mechanism or interference with the Na/K ATPase exchange system. This latter system is probable since the toxin has been implicated in altering membrane bound enzyme systems.

In toxin treated cells, the net sodium ion flux is zero although sodium ion may be absorbed by a substrate dependent pathway whereby sodium ion is absorbed passively with glucose or amino acids which are actively transported across the cell membrane.

If toxin acts directly on adenyl cyclase, one would expect, as in other cyclase mediated systems that the results would be rapid. However, with cholera toxin a time delay is observed and enzymic activity increases after 15 minutes, after which there is a close correspondence between enzymic activity and fluid secretion, The toxin causes a large increase in basal activity with no further synthesis of the enzyme. Since washing does not dislodge the toxin, it may be considered that a permanent change to the enzyme has occurred which does not alter its properties except the V_{max}.

Toxin may stimulate adenyl cyclase in four ways;

i) directly
ii) by conversion to an activator
iii) by release or synthesis of an activator and
iv) by removal of an inhibitary factor or provision of a rate-limiting one.

Toxin probably does not stimulate adenyl cyclase directly due to the observed time lag. If toxin is converted to an activater, this may explain why it is detected in the supranuclear portion of cells using immunofluorescent techniques. In such circumstances, the toxin would be acting as a hormone.

If an activator is synthesised or released, its effects would have to be permanent. Although cycloheximide blocks protein synthesis and fluid production in toxin treated cells, it has no effect on lipolysis and glycogenolysis which toxin also stimulates. This eliminates a polypeptide activator. A prostaglandin could be the

activator although effects due to such a compound are not permanent and even after maximum stimulation by toxin, adenyl cyclase still remains sensitive to PGE_1, which decreases intracellular cAMP concentrations.

Since adenyl cyclase may be stimulated by hormones, it may be considered as normally in a repressed condition. Phospholipids have been shown to be involved in the hormonal stimulation of adenyl cyclase and both adenyl cyclase and the Na/K ATPase are phospholipid dependent.

Leitch et al [31] have demonstrated that toxins liberated from V. cholerae include a neuraminidase and several phospholipases. The neuraminidase causes loss of sialic acid residues from gangliosides present in the cell membrane and loss of these residues may be responsible for the inhibition of the Na/K ATPase. Phospholipases disrupt the membrane and may cause the release of phospholipids intracellularly, one of which may be the rate-limiting factor of adenyl cyclase. Indeed, cholera toxin itself has been shown to possess phospholipase activity and may be a prophospholipase or an activator of an existing membrane-bound phospholipase.

In support of the hypothesis that activity of the adenyl cyclase may be controlled by phospholipids, Levey and Klein [32] have shown that phosphatidyl serine and phosphatidyl inositol restore responsiveness to different hormones on administration to soluble adenyl cyclase in vitro.

van Heyningen [47] has observed that the toxin may be deactivated by gangliosides. This is probably involved in the rapid fixing of the toxin to the mucosal epithelium from where it may direct its effects. It may be postulated that it is the bound toxin that is responsible for the release of the phospholipid which positively modifies the adenyl cyclase.

Bacteria may also exert their pathogenic effects by invading the intestinal epithelium. Sprinz [46] considers that invasive bacteria fall into two classes; those which enter the mucosal cell with little apparent damage eg. Salmonella typhi and those which have a deleterious effect on the cell eg. Shigella dysenteriae.

After ingestion, the shigella bacilli pass to the large intestine

where they attach to the mucosal cells and induce them to ingest them. However, this phagocytosis apparently damages the membrane. Intracellularly, the bacilli multiply and produce a powerful enterotoxin which causes fluid secretion probably in a similar way to cholera toxin. Bacilli spread into adjacent cells eventually killing them. These cells are sloughed off, and due to the loss of the epithelium, ulceration occurs resulting in the passage of bloody purulent stools characteristic of bacillary dysentery. Mucosal cell damage is caused by the lipopolysaccharide endotoxin liberated by all shigella species.

Salmonella species which do not injure the epithelium may elicit two distinct host responses in man. When S. typhimurium is ingested, it causes a predominantly neutrophilic response and subsequent enteritis in a localized region of the intestine. However, S. typhi causes a monocytic response resulting in a bacteraemia and culminating in typhoid fever, which is unique to man. Although there is no evidence for a macrophage toxinogen, a polymorphonuclear toxinogen has been demonstrated in E. coli. It seems plausible that S. typhi may produce a macrophage toxinogen or migration inhibition factor.

Since typhoid fever is unique to man, there is a lack of experimental evidence. The highest incidence of typhoid fever is seen in children in Chile and Egypt on their first exposure to the bacillus. Adults are observed to have a substantial resistance due to previous encounters. In only a few cases does disease develop in these circumstances.

Whether typhoid fever develops when S. typhi is ingested depends on how many bacilli are ingested, and their antigenic content eg. if they possess the Vi capsular antigen. With a characterized strain, Hornick [27], has shown that the ID_{50} was about 10^7 bacilli and that the incubation period and pathogenicity depends on the number ingested. In Zermatt in 1963 10^5 bacilli must have been ingested by each subject assuming the two strains to be of equal virulence and pathogenicity. In the experiment, the ID_{25} was about this number.

S. typhi multiply rapidly in the small intestine, and attach to the mucosal cell which phagocytoses without apparent damage to itself. The bacilli multiply intracellularly and are found in the stools within 24 hours. The organisms pass rapidly through the mucosal cell and are then found in the lamina propria where they are ingested by macrophages. Possession of the Vi antigen may enable the bacilli to remain alive and

multiply inside these phagocytes. Hornick has shown that virulence of the bacillus in humans is apparently doubled by possession of this antigen. The bacteria subsequently escape into the lymphatics, reaching the blood stream via the mesenteric nodes where they multiply. Various organs are infected during this first bacteraemia including the liver, gall-bladder and bone-marrow. After 7-10 days a second bacteraemia begins, the onset coinciding with pyrexia and other symptoms including abdominal pain, and headache. The gall-bladder is of particular importance as a harbour for the bacteria since they may reside here for many years and subsequently be released into the intestine and hence the faeces causing infection to others, and possibly the carrier. On second exposure to the bacillus, the intestine inflames and is invaded by polymorphs. Subsequent ulceration occurs due to the damage to the epithelium.

S. typhi produces an endotoxin which may cause the release of an endogenous pyrogen from polymorphs and monocytes. This would act on the thermo-regulatory centre of the hypothalamus, producing the pyrexia. In mice, the endotoxin stimulates macrophage respiration, phagocytosis and bactericidal activity.

Different strains of the same bacterium may produce disease in different ways. Infantile gastro-enteritis due to E. coli may be caused by production of an enterotoxin, acting similarly to cholera toxin [17,21]. This disease occurs in infants due to lack of acquired specific immunity to the toxin. Smith and Halls [45] have demonstrated that enterotoxin production is conferred by a plasmid which is not species specific since it may be transferred from S. typhimurium to E. coli. Other strains of E. coli appear to be of an invasive nature like shigella or salmonella species.

Another mechanism by which bacteria may cause disease in the gut include an autoimmune reaction by the host. Previously ulcerative colitis was thought to be due to a particular organism (Cooke [4]). However, it appears that E. coli 014 strain and the colon possess a common antigen and that in reacting to the bacillus the host also reacts to his own tissue.

Other bacteria which may produce pathology in the gut include Staphylococcus aureus which produces an enterotoxin, and Proteus morganii which may cause summer diarrhoea in infants. Ingested Cl. botulinum

elaborates a very potent neurotoxin which is rapidly lethal.

Bacteria may be responsible for cancer of the large bowel. Hill et al [26] have demonstrated that the diet determines the bacterial population of the gut and that in diets with a high fat intake there are proportionately more anaerobes ie. bacteroides than in a low fat diet where aerobes like streptococci and the enterobacteria predominate. Anaerobes metabolize steroids more actively than aerobes and in subjects with a high fat diet, there are about 100 times more bacteria able to dehydroxylate cholic acid at the 7α position than in low fat diet controls. There is a higher incidence of colon carcinoma where the faecal concentration of dihydroxycholanic acids like deoxycholic acid is increased. Deoxycholate is a weak carcinogen but over 50 years about 1300 g. pass through the gut. Hence, it need not be very active to account for 18 cases per 100,000 in 45-64 years old age group. Bacteria are certainly able to methylate dehydronorcholene to the potent carcinogen 20 methyl cholanthrene but it is debatable if they synthesise dehydronorcholene from deoxycholic acid. However, this remains a possibility.

Burkitt [3] has added that the decreased fibre intake particularly cellulose in typical Western diets with high fat intake causes an increased time for the oral-anal passage due to colonic stasis. This could result in higher concentrations of deoxycholate and possibly other carcinogens for longer periods in the intestine. Combination of these factors may be responsible for bowel tumours.

Viruses are found in the gut [30] and nearly all are picornaviruses. Although many of these are apparently harmless and replicate in the gut some of these enteroviruses eg. poliovirus are important pathogens outside the gut. An as yet unidentified virus may be the cause of "epidemic vomiting". This virus is host specific for man and may induce transient immunity. However, due to the obvious difficulties in culture technique, little is known of this small virus. Other enteroviruses include echoviruses and coxsackieviruses which are both responsible for aseptic meningitis.

A considerable amount of work remains to be done to understand exactly how bacterial enterotoxins act. Similarly the role of viruses in intestinal pathology is poorly understood.

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